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White Light–Emitting Diodes (LEDs) at Domestic Lighting Levels and Retinal Injury in a Rat Model

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Running title: LED light source-induced retinal injury

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Abstract

Background: Light-emitting diodes (LEDs) deliver higher levels of blue light to the retina compared to conventional domestic light sources. Light-induced retinal injury due to chronic exposure to relatively low-intensity (750 lux) light in a rodent model, compared to the intensities examined in previous studies (2000-10000 lux), has not been previously assessed with LEDs.

Objective: We examined LED-induced retinal neuronal cell damage in the Sprague-Dawley rat model through functional, histological, and biochemical measurements.

Methods: Blue LEDs (460 nm) and full-spectrum white LEDs coupled with matching compact fluorescent lights were used for exposure treatments. Electroretinogram, hematoxylin and eosin (H&E), immunohistochemical (IHC) stain, and transmission electron microscopy (TEM) were used for pathological examinations. Free radical production in the retina was measured to determine the oxidative stress level.

Results: H&E staining and the TEM study revealed apoptosis and necrosis of photoreceptors, which indicated blue-light induced photochemical injury of the retina. Free radical production in the retina increased in the LED exposure groups. The IHC stain demonstrated that oxidative stress was associated with retinal injury. While LED group demonstrated serious retinal light injury, compact fluorescent lamp (CFL) group showed moderate to mild injury from the exposure experiments.

Conclusion: The results raise questions related to adverse effects on the retina from chronic LED light exposure compared to current lamp sources that have less blue light. Our results suggest a precautionary approach may be advisable with regard to the employment of blue-rich "white" LEDs for general lighting.

Introduction

Artificial lighting is a basic element in modern society; however, the potential health risks caused by light pollution have increased with the development of more sophisticated lighting technology (Chepesiuk 2009). Among the wide variety of artificial lighting selections, light-emitting diodes (LEDs) emit higher levels of blue light compared to conventional light sources. This is also the first time that humans have experienced such extensive blue-light exposure (Behar-Cohen et al. 2011). From an environmental health perspective, retinal light injury and the potential risks for chronic exposure from using LEDs as a domestic light source require assessment before further development of this important, energy-saving technology.

Because LED (or solid-state) lighting sources are designed to emit all energy within the wavelength range of human vision, it is the most energy-efficient commercially manufactured light; however, many current "white-light" LED designs emit much more blue light than conventional lamps, which has a number of health implications, including disruption of circadian rhythms (Holzman 2010). The most popular LED lighting product, a phosphate-conversion (PC) LED, is a LED chip that emits blue light passing through a yellow phosphor-coating layer to generate the ultimate white light (Spivey 2011). Although the white light generated from LEDs appears normal to human vision, a strong peak of blue light ranging from 460 to 500 nm is also emitted within the white light spectrum and corresponds to a known spectrum for retinal hazards (Behar-Cohen et al. 2011). Some epidemiological studies have suggested that short-wavelength light exposure is a predisposing cause for age-related macular degeneration (AMD) (Wu et al. 2006). Animal models have also been used to determine that excessive exposure to blue light is a critical factor in photochemical retinal injury targeting photoreceptors and the retinal pigment

epithelium (RPE) (Hafezi et al. 1997).

Photochemical retinal injury resulting from a cumulative effect is caused by free radicals generated from retinal tissue through continuous light exposure (Dong et al. 2006). When exposure surpasses the protective capability, unfavorable free radicals and reactive oxygen species may form (Wu et al. 2006). This enhances the oxygenated products and provides conditions favorable for photodynamic damage of photoreceptors and other retinal tissues (Beatty et al. 2000). However, the wavelength-dependent effect and its influences on white LED light-induced retinal degenerations remain unknown.

Retinal light injury was studied intensively after Noell (Noell et al. 1966) first described the retinal damage caused by environmental fluorescent light exposure and numerous studies have reported that high-intensity blue light causes acute retinal injury (Ham et al. 1976). However, few studies have focused on retinal injury caused by exposure to relatively low-intensity blue light under chronic exposure conditions (Peng et al. 2012). The composition of the white-light spectrum differs among LED products, and their light qualities change over time. Although it is robust in the beginning, a PC-LED progressively releases more short-wavelengths (blue light) when LED lumen depreciation occurs because of phosphor degradation. The quality of the light deteriorates after the lights pass the 70% lumen maintenance level (L70) (U.S. Department of Energy 2009). These characteristics suggest that a white LED can cause more blue light exposure than other domestic lighting sources. Considering that cumulative exposure to blue light has been argued to accelerate aging of the retina and may play an etiological role in age-related macular degeneration (AMD) (Behar-Cohen et al. 2011), domestic lighting with high blue light requires further examination to determine its potential retinal effects.

We hypothesized that LED chronic exposure may induce retinal photochemical injury. This study was performed in a rat model and the retinal neuronal cell damage caused by oxidative stress was examined. Functional, histological, and biochemical measurements were applied to identify the biomarkers for retinal light injury.

Methods

Animals and rearing conditions

The experimental protocols are detailed in Supplemental Material. In total, 120 adult male Sprague–Dawley rats were purchased from BioLasco Taiwan Co., Ltd at 8 weeks of age and stored in a dark environment for 14 days to clear the light exposure effect from their previous rearing environment. Twelve normal rats served as controls without exposure, and the other 108 rats received programmed light exposure, as shown in Figure 1. All animals received food and water *ad libitum*. The use of rats in this study conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. The animals were treated humanely and with regard for alleviation of suffering.

Light source

As shown in Figure 2. Single-wavelength blue LEDs (460 ± 10 nm) and blue LED with yellow phosphor-converted (PC) white LEDs were custom made for the exposure experiments. The PC-LED had a correlated color temperature (CCT) of 6500 K. One group of white compact fluorescent lamps (CFL) also matched the CCT at 6500 K (Chuanshih, ESE27D-EX, Taiwan), whereas the other group of yellow CFL was set at 3000 K (Chuanshih, ESE27L-EX, Taiwan). Each light source was programed for 40 measurements in an integrating sphere. The spectrum distributions and total intensities for all light sources were tested by the Industrial Technology

Research Institute of Taiwan, a Certification Body Testing Laboratory (CBTL).

Light exposure. As shown in Figure 1. The animals were divided into 4 groups, and each rat was stored in an individual transparent cage with a dimension of 45 x 25 x 20 (cm). Each cage was placed in the center of a rack shelf with dimensions of 75 x 45 x 35 (cm). The light sources were set on the top of each shelf and were measured 20 cm away from each source to acquire the common domestic luminance level at 750 lux. After 14 d of dark maintenance, the light exposure started at 6:00 PM of Day 15 with the total exposure duration ranging from 3, 9, to 28 d under 12 hr dark/12 hr light cyclic routines. The animals were sacrificed for analysis after light exposure. However, a special treatment for 32 animals was performed, 8 from each group were returned to a dark environment for 14 d of recovery after 28 d of exposure. The objective of the recovery stage was to allow for possible removal of necrotic photoreceptor cell debris.

Electroretinography (ERG)

ERG was performed as described previously with modification (Schatz et al. 2012). In brief, retinal electrical responses were recorded before and after light exposure using ERG (Acrivet, Hennigsdorf, Germany). Alcaine (0.5%) (proxymetacaine hydrochloride; Alcon Pharmaceuticals Ltd, Puurs, Belgium) was applied for local anesthesia. Each 20-milli-second flash was provided by a 4 W LED (1 mV), and the illumination was set at 2.5 log $cd \cdot s/m^2$ for Scotopic ERG response. The weighted average of 10 stimulations was computed by the program to produce the final detection values.

Hematoxylin and eosin (H&E staining)

Retinal histology was performed as described previously with modifications (Collier et al. 2011) after 9, 28, 28+14 d of light exposure. In brief, after pretreatment, paraffin sectioning was

performed, the sections were dehydrated in ethanol, infiltrated in xylene, and embedded in paraffin. Radial 5 µm sections were stored at 48°C. Histologic analysis included quantification of the outer nuclear layer (ONL) and retina morphology alteration using a light microscope. The midsuperior aspect of the retina was examined for all histological analyses in this study.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)

The TUNEL assay was performed using a FragEL^{TM} DNA fragmentation detection kit (Calbiochem, Darmstadt, Germany) following the standard protocol to detect apoptotic cells after 9 d of light exposure. The tissues were counter-stained with DAPI, and the DNA strand breaks were labeled with fluorescein FITC-Avidin D. Sections were visualized on a fluorescent microscope over the entire retina excluding the RPE layer (Nikon Instruments Inc., NY, USA). The number of TUNEL-positive cells for each section was counted by Image-Pro Plus software (v.6.0).

Immunohistochemistry (IHC)

Immunohistochemistry was performed as described previously (Collier et al. 2011; Fang et al. 2013) after 9 d of light exposure. In brief, cryosections of the retina samples were incubated overnight at 48°C with specific primary antibodies. Three antibodies were used for detection as follows: 8-hydroxy-2'-deoxyguanosine (8-OHdG) (1:50, JAICA, Japan) for DNA, acrolein (1:200, Advanced Targeting Systems, San Diego CA USA) for lipids, and nitrotyrosine (1:200, Abcam, Millipore Billerica, MA USA) for proteins. The number of IHC-positive cells for each section was counted by Image-Pro Plus software (v.6.0).

Transmission electron microscopy (TEM) analysis

TEM was performed as described previously (Hafezi et al. 1997) after 9 d of light exposure.

In brief, sections were coated with thin copper grid-film and placed in a vacuum chamber for scanning. The specimens were examined using TEM with a high-resolution instrument at 80 kV (JEOL JEM-1400, Japan).

Free radical assay (reactive oxidative species)

Measurement of reactive oxygen species in the retina was performed as described previously (Fang et al. 2013) after 3 or 9 d of light exposure. In brief, reactive oxygen species were quantified after adding the enhancers lucigenin (bis-N-methylacridiniumnitrate) to the chemiluminescence analyzer (Tohoku CLA-FS1, Japan). The stimulated superoxide anion (O_2^-) and total oxidative products were captured every 10 s and computed for 7 min after 1 min of baseline detection.

Statistical analysis

Data were presented as the mean ± SD unless otherwise stated. Data were evaluated using analysis of variance (ANOVA) with Tukey post hoc tests to show differences between the groups. A p value less than 0.05 was considered to be statistically significant.

Results

Electrophysiological response shows photoreceptor cell function loss

The representative ERG response curves of rats are shown in Figure 3A. The normal retina showed a high b-wave peak, but the injured retina curved a low b-wave peak as a result of cell function loss. As shown in Figure 3B. Two LED groups and the white CFL group all demonstrated a significant decrease of b-wave amplitude at day 9 and day 28 after light exposure (ANOVA followed by Tukey post hoc test $p < 0.001$). The b-wave amplitude of the yellow CFL

group did not decrease significantly at day 9; however, it had 21% of decrease at day 28 after light exposure. The data from each of the four exposure groups was not statistically different at 28+14 d as compared to 28 d of exposure, and this trend was also applied to the H&E staining results (data not shown). No significant development was found after 3 d of light exposure, and therefore data were not shown as well.

Retinal histology–H&E staining showing layer damages

As shown in Figure 4a-b. White LED light exposure can lead to morphologic alterations in the rat retina. The group that was exposed to 750 lux white LED light for 28 d exhibited the adverse effect of light exposure including the pyknotic photoreceptor nuclei (arrow), swelling of the inner segment (arrow head), and a disorganized outer segment (asterisk). As shown in Figure 4c-f. The ONL thickness of white and blue LED groups decreased significantly at day 9 and day 28 (data not shown) after light exposure (ANOVA followed by Tukey post hoc test $p < 0.01$), whereas, the ONL thickness of the white and yellow CFL groups did not decrease significantly at day 9 after light exposure.

Apoptosis Detection - TUNEL staining detects nuclear apoptosis

The retinal TUNEL stains are shown in Figure 5. Light exposure induced significant retinal cell apoptosis in all groups. However, more apoptotic cells were shown in the retina of the LED groups than in the retina of the CFL lamp groups after 9 d of exposure (ANOVA followed by Tukey post hoc test $p < 0.001$ for LED groups; $p < 0.01$ for CFL groups).

TEM demonstrations on the cellular injury

As shown in Figure 6 (samples were taken after 9 d of white LED light exposure). Nucleolus damage of photoreceptors occurred after exposure including early stage of nucleolus

condensation (6b), karyolysis (6c), pyknosis (6d-e), and karyorrhexis (6f). Another crucial observation of photoreceptor damage included disruption of the inner and outer segments, which is shown in Figure 6g-l.

Immunohistochemistry (IHC) staining results indicating retinal light injury

Oxidative damage results in adducts on macromolecules that can be detected by immunostaining. The antibodies that specifically recognize these adducts provide evidence of the oxidative damage. Three antibodies were used to detect cell conditions in these experiments after 9 d of light exposure, including acrolein for lipid recognition (Figure 7A), 8-OHdG for DNA detection (Figure 7B), and nitrotyrosine for protein identification (Figure 7C). The results show that LED groups exhibit higher fluorescence intensity with 8-OHdG, acrolein and nitrotyrosine in ONL (ANOVA followed by Tukey post hoc test $p < 0.001$ for LED groups) and that the fluorescent lamps induced lower fluorescence intensity of 8-OHdG, acrolein and nitrotyrosine in ONL.

Oxidative Stress -- superoxide anion O_2^- shows the injury

As shown in Figure 8A. Lucigenin-stimulated superoxide anion (O_2^-) and total oxidative products were computed for all groups. After 3 d of blue LED light exposure, the retina O_2^- exceeded 60000 in 8 min, the white LED group exhibited a high total count close to 40000, and the fluorescent groups accumulated smaller total counts from 20000 to 30000. However, the plot exhibited an opposite trend when the exposure duration was increased to 9 d (Figure 8B). This result suggests that retinal oxidative stress may be induced by light exposure in the early stage.

Discussion

Retinal light damage depends on the duration of exposure and light level reaching the retina

(retinal irradiance). The pathological process is also wavelength dependent (Organisciak and Vaughan 2010). The experiment results of the present study clearly demonstrate that exposure to LED light can induce retinal damage as evidenced by the functional ERG study, IHC, TUNEL and TEM examinations in an albino rat model. Our results also demonstrated that this retinal damage could be related to the blue light-induced oxidative stress within the retinal tissues, as evidenced by the ROS generated in the retina after LED light exposure.

The ERG results clearly indicate functional loss in the retina after LED light exposure. The white and blue LED group demonstrated a significant decrease in the b-wave amplitude at day 9 and day 28 after light exposure. The morphological results show that cyclic white LED light exposure may cause outer retinal damage within 9 d and may cause further deterioration when the exposure duration is extended. ONL, which is usually 12-14 rows of nuclei in normal Sprague-Dawley rats at 2-3 months, was reduced to approximately 4-5 rows. OS and IS were absent, and the RPE appeared to be damaged or missing. However, yellow fluorescent light exposure induced less damage within the photoreceptor, as shown in Figure 4f. Therefore, above functional and morphological results indicate that the wavelength and the spectral power distribution (SPD) rather than total light irradiance, are crucial risk factors that contribute to photochemical retinal injury. The results of the present study also suggest that LED light-induced cell death may occur through the intrinsic apoptotic pathway under oxidative stress. However, Sliney calculated that for the same lamp brightness, the retinal irradiance in the rat eye would be at least 60% greater than experienced by the human retina (Sliney 1984). The exposure started at 6:00 PM to match the nocturnal activity pattern may also enhance the light damage susceptibility. Therefore, the careful development of an action spectrum for LED light damage

remains an important research goal.

The retina is one of the highest oxygen-consuming tissues in the body and is sensitive to oxidative stress (Yu and Cringle 2005). Oxidative stress is the crucial risk factor for photoreceptor degeneration, which is caused by the generation of toxic reactive oxidative species within retinal tissue. The retina contains enzymes involved in detoxification or synthesis, particularly in OS or RPE (Newsome et al. 1990). This report is an initial study to compare the phototoxicity with the fluorescent lamps and typical white LEDs. It is clear that typical white LED lights carry higher energy that exceeds the threshold of this stress-induced protection mechanism and results in severe damage to the outer retina. Therefore, some companies are increasing the market segments of lower color-temperature LEDs for domestic lighting selection.

Photochemical damage is the major cause of low-intensity chronic exposure light-induced injury. Noell indicated that the direct action of light on photoreactive molecules within the damaged cell causes primary damage. Secondary damage, which follows the primary event, can either continue the damaging process in the same cell or expand to other cells (Noell 1980). The main concern is that light damage involves oxidative events (Lohr et al. 2006). As shown in Figure 1. We used several exposure durations in the study to analyze cause and effect in a temporal manner. The results of the present study show that LED lights carry energy that is strong enough to generate oxidative stress. The experimental results are consistent with the observation by Noell; that is, retinal neuronal cell DNA levels and ERG b-wave estimates of photoreceptor cell loss correlate in light-damaged rats. Oxidative stress is responsible for light damage pathogenesis, especially when light is sufficient to damage over 80% of photoreceptor cells detected by non-recoverable ERG b-waves. Furthermore, the histological analysis showed that

most cell death does not occur immediately after light exposure. The damaged retinal neuronal cells may lose function, yet appear on the retina layers with oxidative modified lipids, nucleic acids, and proteins.

Conclusions

LEDs are expected to become the primary domestic light sources in the near future. Certain amount of LED light exposure may cause retinal damage, and the animal model provides comparative measures of damage from different commercial light sources. Albino rats are commonly used for retinal light injury experiments (Collier et al. 2011). Although 14 d dark maintained albino retinas are more susceptible to light-induced damage than normal pigmented retinas, our results show that the SPDs of white LEDs contain a major fraction of short-wavelength light that causes irreversible retinal neuronal cell death in rats. Furthermore, this model clearly demonstrates that the SPD of white LEDs now being introduced for domestic lighting pose a theoretical risk compared to CFLs (or, incandescent lamps with little blue). When analyzing blue-light hazards, we cannot exclude the risk of chronic effects from daily exposure because photochemical damage may not induce an acute syndrome; instead, blue light exposure may cumulatively induce photoreceptors loss.

Regardless of whether the initial damage is caused by a photochemical effect, LED light damage is dependent on wavelength and duration. The entire retinal neuronal cell is affected, regardless of whether the injury is localized in the outer segment, mitochondria, or other subcellular organelles. Because these results show that LED domestic light source illuminance levels can induce retinal degeneration in experimental albino animals, the exact risks for the pigmented

human retina require further investigation.

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Figure Legends

Figure 1. Time frame of the experimental design. ROS: Reactive oxygen species. ERG: Electroretinography. H&E: hematoxylin and eosin staining. TEM: Transmission electron microscopy. IHC: Immunohistochemistry. TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling stain. After 14 days of dark maintenance, the rats were divided into 4 groups and exposed to different light sources. During the exposure and recovery stages, certain analytical techniques (red boxes in the figure) were performed at the end of various exposure durations.

Figure 2. Light source spectral power distribution (SPD) curves. (A) Single-wavelength blue light LED peaked at 460 nm with 0.1 W/nm in radiometric units. (B) Yellow phosphor-converted white LED to exhibit CCT at 6500 K; the first peak appeared at 460 nm with 0.028 W/nm showing the blue content and the second peak with a bell shape presenting a large portion of yellow content. (C) Compact fluorescent lamp with CCT at 6500 K showing several sharp peaks across the spectrum; the blue peak is relatively shorter than the yellow or red peaks, and the full width at half maximum (FWHM) is smaller than that in (A) and (B). (D) Similar to the condition in (C) with CCT at 3000 K, the highest peak is presented in yellow. Although all light sources contain blue light peaks, the area under the curve variation leads to a total intensity difference.

Figure 3. ERG responses after light exposure. Both LED groups demonstrated a significant decrease of b-wave amplitude at day 9 and day 28 after light exposure. The fluorescent lamp groups developed severe loss of b-wave amplitude until 28 d of light exposure. n = 3 for controls, n = 3 for 3 days of exposure groups, and n = 8 for each exposure group at each time of exposure (Curve scale: amplitude = 250 μV and stimulation = 50 msec). (**, *** $p < 0.01, 0.001,$

respectively, compared to the “normal” group by ANOVA with the Tukey post hoc test).

Figure 4. Retinal light injury after 9 d or 28 d of exposure analyzed by H&E staining. GCL: ganglion cell layer. INL: inner nuclear layer. ONL: outer nuclear layer. IS: inner segment. OS: outer segment. *RPE: the retinal pigment epithelium (usually next to the OS layer) is detached and cannot be found within this scope. (A) (a) Normal retina layers, and (b) light exposure-induced retinal injury, including the absence of photoreceptors and INL degeneration. (B) The ONL thickness of the LED groups decreased significantly at day 9 and day 28 after light exposure, whereas the ONL thickness of white and yellow CFL groups did not decrease significantly at day 9 after light exposure. Both blue (c) and white LED (d) light exposure caused the disappearance of photoreceptors; the white CFL group (e) exhibited distortion of the OS and ONL; and the yellow CFL group (f) exhibited less movement in each layer. n = 3 for controls and n = 8 for each group after 9 or 28 days of exposure (** indicates $p < 0.01$, compared to the “normal” group by ANOVA with the Tukey post hoc test; scale bar = 50 μm).

Figure 5. Light-induced retinal cell apoptosis tested by TUNEL labeling. GCL: ganglion cell layer. INL: inner nuclear layer. ONL: outer nuclear layer. RPE: the retinal pigment epithelium. The damaged retina cells correspond to the positive labeling. (A) The result shows that more apoptotic cells (arrows) appear in the retina of the LED groups than that of the CFL groups after 9 days of light exposure. (B) The LED groups exhibit higher fluorescence intensity. n = 3 for controls and n = 8 for each exposure group (**, *** $p < 0.01$, 0.001, respectively, compared to the “normal” group by ANOVA with the Tukey post hoc test; scale bar = 50 μm).

Figure 6. Retinal cellular injury studied by TEM. The photoreceptor nucleolus damage after LED light exposure result in (A) ONL nuclear deformations (arrows) shown as (a) normal ONL

nucleus; (b) nucleolus condensation; (c) karyolysis; (d and e) pyknosis; (f) karyorrhexis. (B) Photoreceptor deformations and (g) normal photoreceptor, IS and OS; (h and i) showing minor disruption; (j, k, and l) and IS disappearance followed by OS shrinkage and the formation of several small round shapes (scale bar = 2 μm for g, h, and k; scale bar = 1 μm for the rest of others). n = 3 for controls and n = 5 for white LED group after 9 days of exposure. Samples were selected to show their representativeness and photographs were taken from different samples.

Figure 7. Retinal light injury labeling after 9 d of exposure by IHC. (A) Acrolein was used to detect the lipid adducts on macromolecules; (B) 8-OHdG was used to detect the DNA adducts; and (C) Nitrotyrosine was used for protein adduct recognition. The result shows LED groups exhibit higher fluorescence intensity on ONL, and the fluorescent lamp groups respond to lower fluorescence intensity on ONL. n = 3 for controls and n = 8 for each exposure group (*, **, *** $p < 0.05, 0.01, 0.001$, respectively, compared to the “normal” group by ANOVA with the Tukey post hoc test; scale bar = 50 μm).

Figure 8. A reactive oxygen species assay after 3 d and 9 d of light exposure. CL: chemiluminescence. (A) After 3 d of blue LED light exposure, the lucigenin-stimulated superoxide anion (O_2^-) exceeded 60000 in total count; the white LED group had a high total count close to 40000; and the fluorescent groups accumulated less total counts from 20000 to 30000, whereas normal rats exhibited only a count of approximately 1000. n = 3 for controls and n = 3 for each exposure group. (B) After 9 d of exposure, the O_2^- total count for the blue LED light group decreased to 8000; the white LED light group decreased to 18000; and both fluorescent light groups remained at the same level at 20000 to 30000. n = 3 for controls and n = 8 for each exposure group (**, *** $p < 0.01, 0.001$, respectively, compared to the “normal”

group by ANOVA with the Tukey post hoc test).

Figure 1

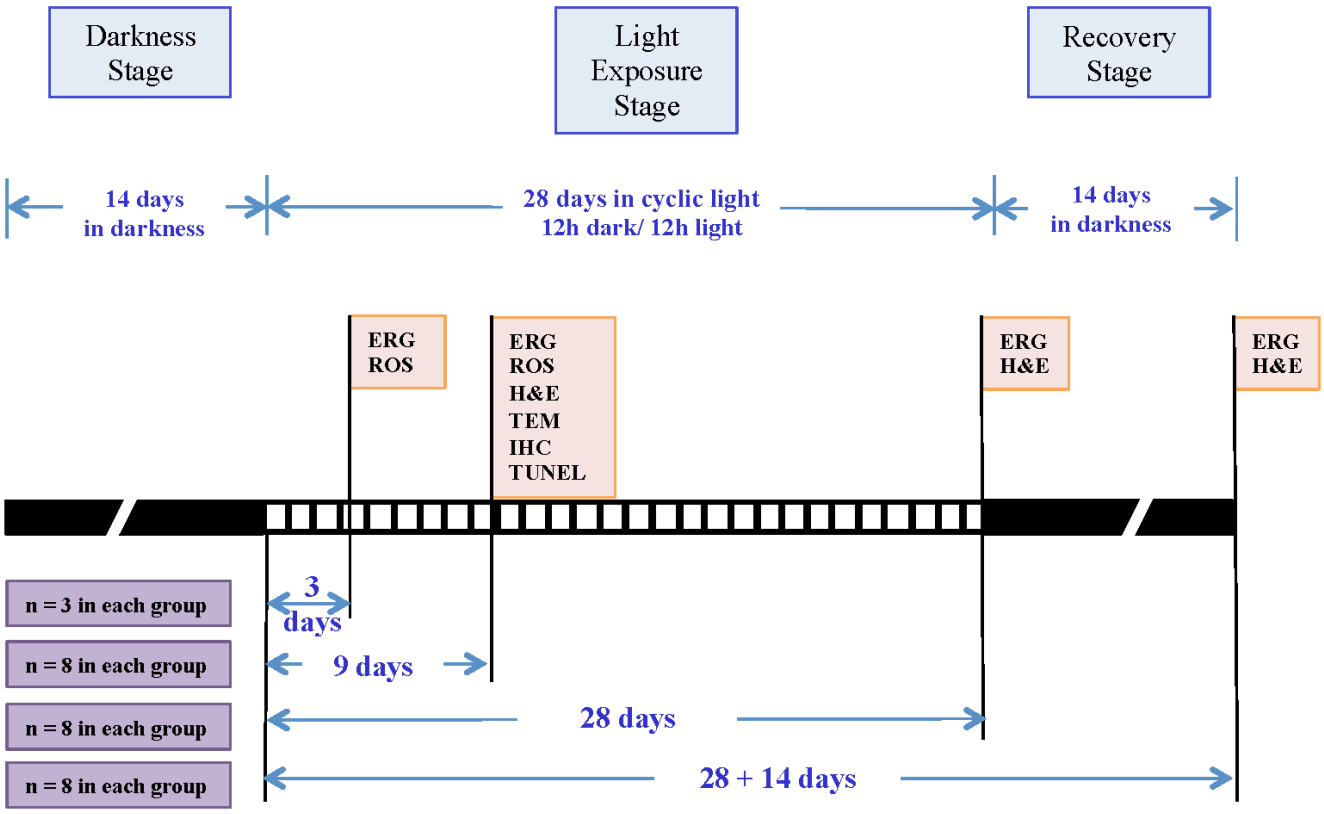
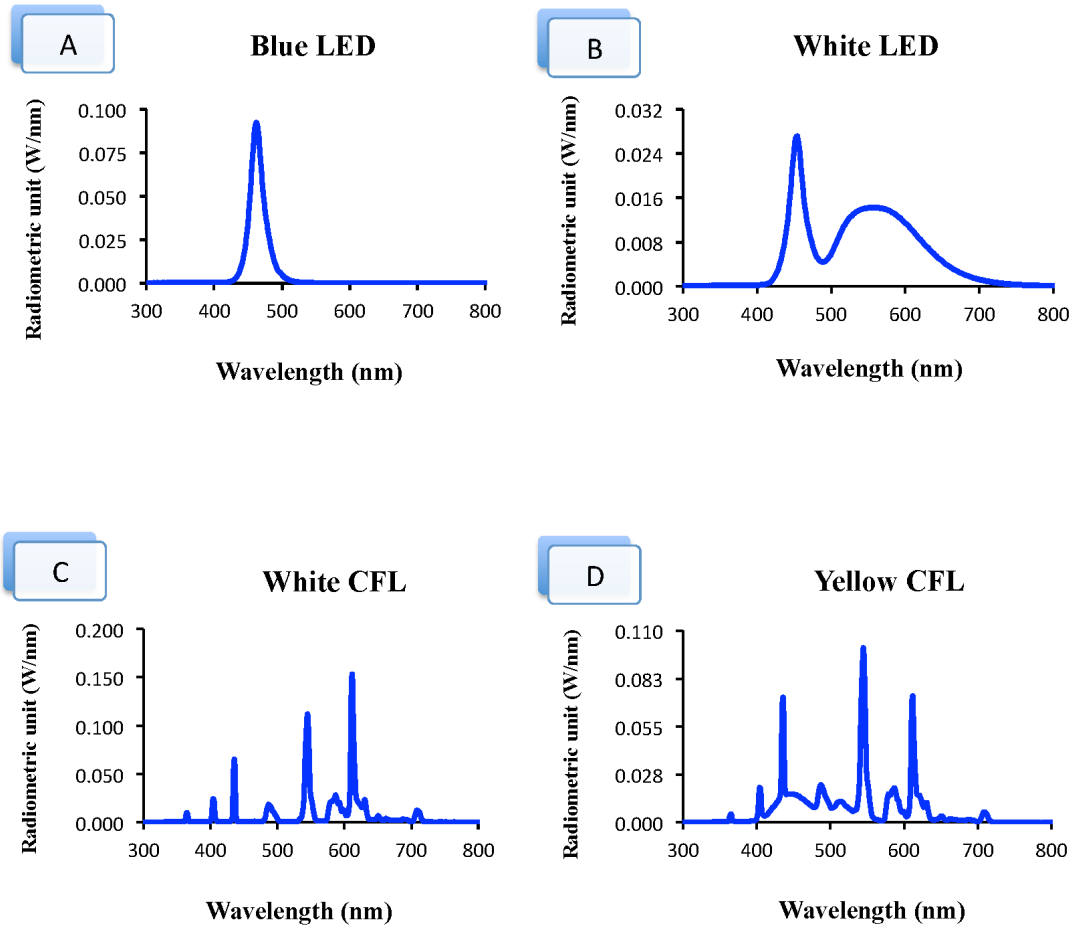

Darkness Stage
Light Exposure Stage
Recovery Stage
14 days in darkness
28 days in cyclic light 12h dark/ 12h light
14 days in darkness
ERG ROS
ERG ROS H&E TEM IHC TUNEL
ERG H&E
ERG H&E
n = 3 in each group
n = 8 in each group
n = 8 in each group
n = 8 in each group
3 days
9 days
28 days
28 + 14 days

Figure 2


A
Blue LED
Radiometric unit (W/nm)
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300
400
500
600
700
800
Wavelength (nm)

Figure 3

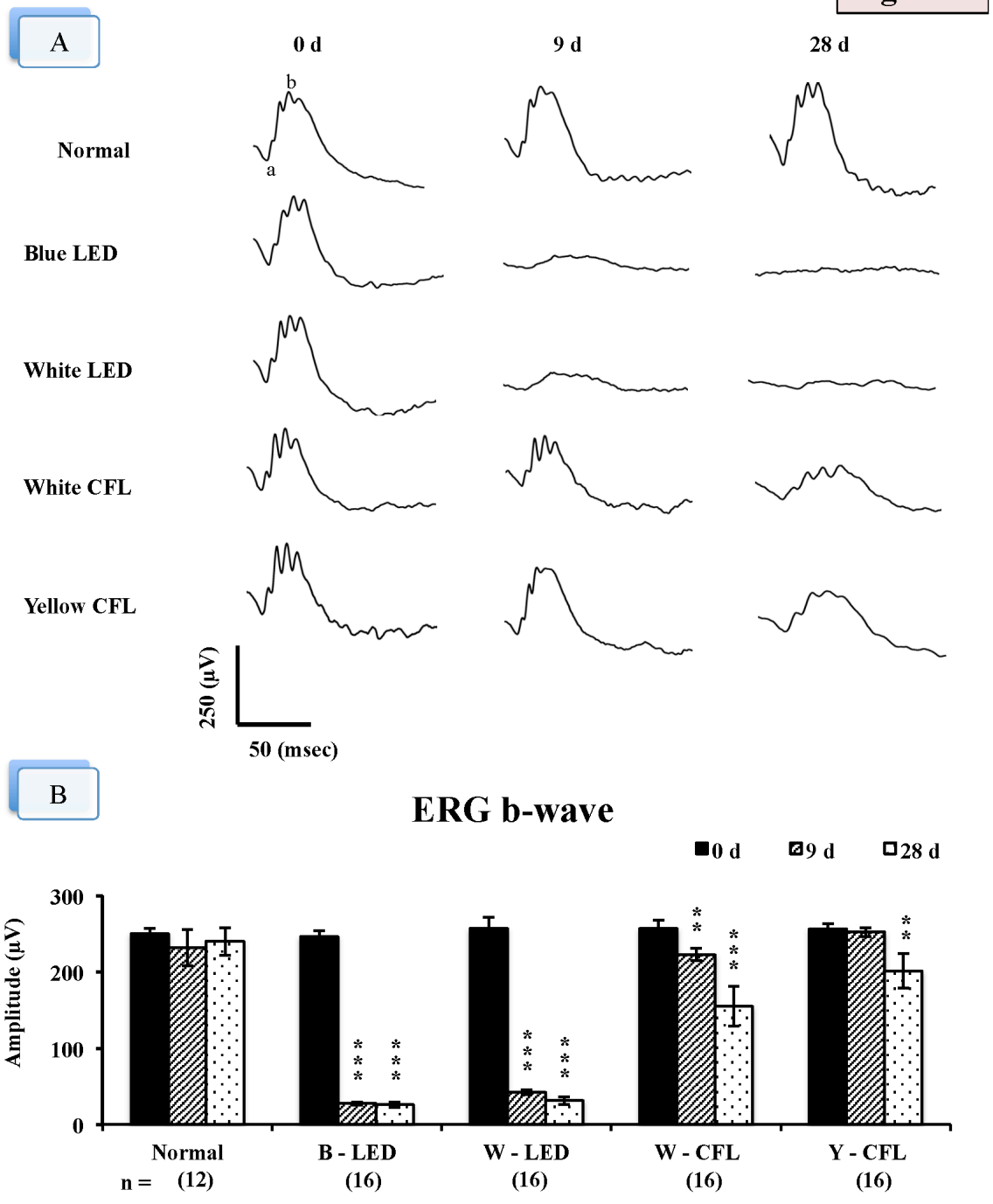

A
0 d
9 d
28 d
b
a
Normal
Blue LED
White LED
White CFL
Yellow CFL
250 (μV)
50 (msec)
B
ERG b-wave
0 d
9 d
28 d
Amplitude (μV)
300
200
100
0
Normal
B - LED
W - LED
W - CFL
Y - CFL
n = (12) (16) (16) (16) (16)

Figure 4

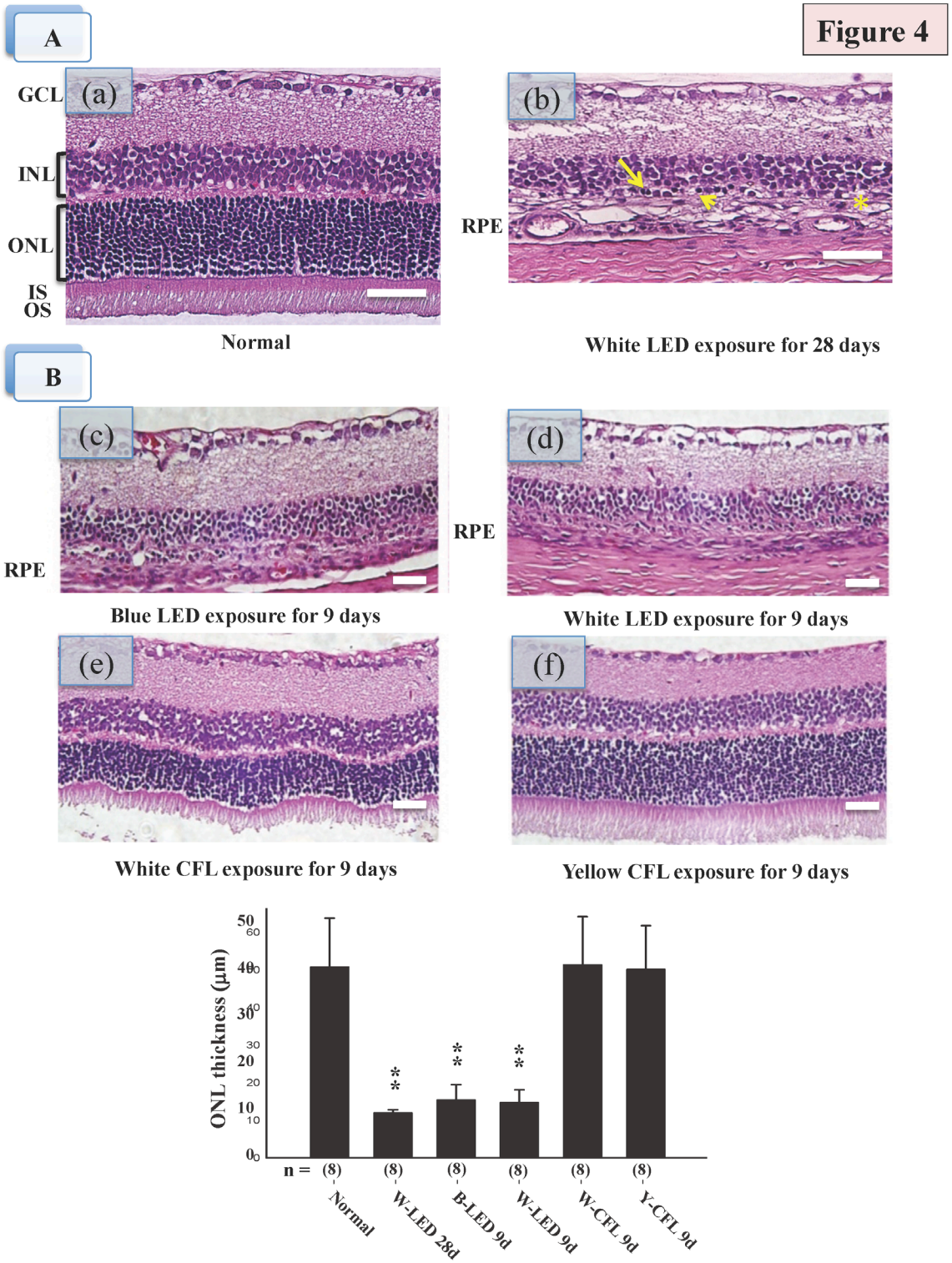
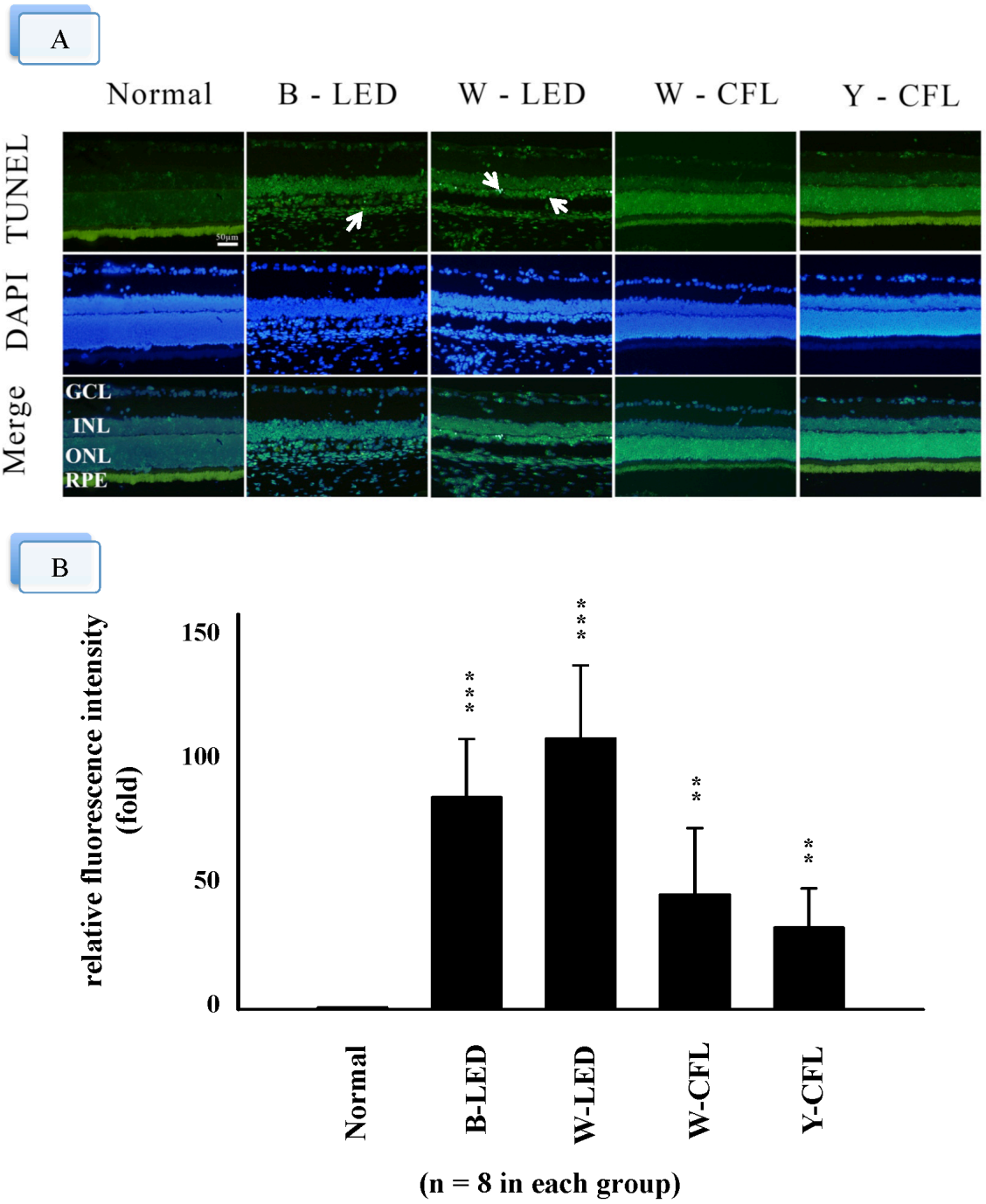
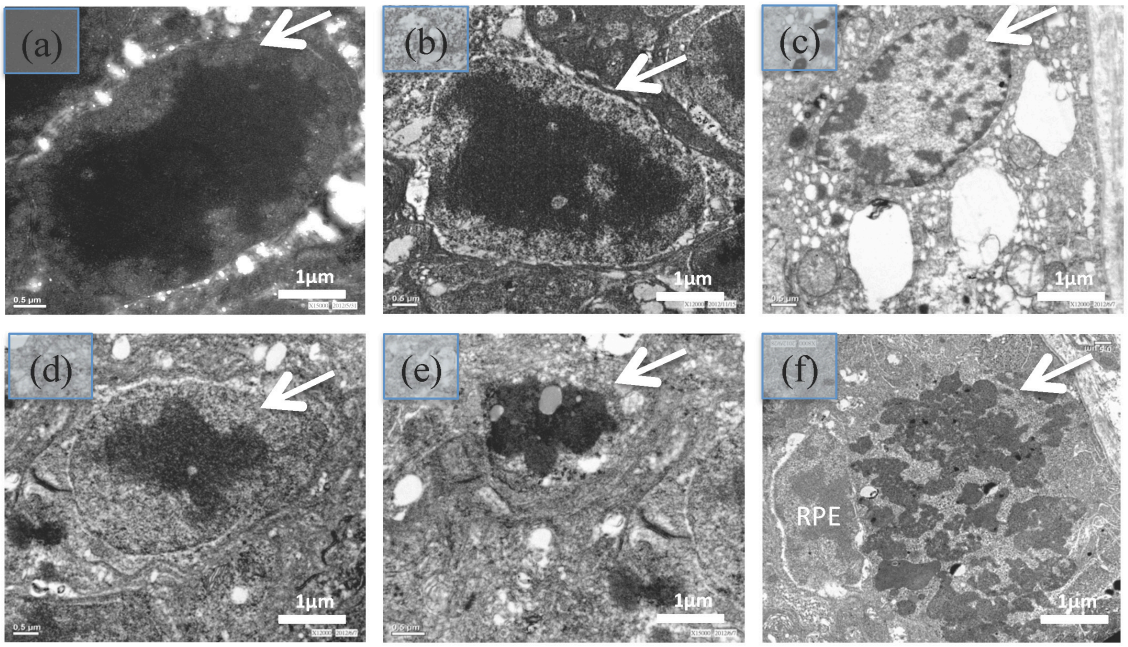

A
(a)
GCL
INL
ONL
IS
OS
Normal
(b)
RPE
White LED exposure for 28 days
B
(c)
RPE
Blue LED exposure for 9 days
(d)
RPE
White LED exposure for 9 days
(e)
White CFL exposure for 9 days
(f)
Yellow CFL exposure for 9 days
ONL thickness (μm)
50
40
30
20
10
0
n = (8) (8) (8) (8) (8) (8)
Normal
W-LED 28d
B-LED 9d
W-LED 9d
W-CFL 9d
Y-CFL 9d

Figure 5


A
Normal
B - LED
W - LED
W - CFL
Y - CFL
TUNEL
DAPI
Merge
GCL
INL
ONL
RPE
50μm
B
relative fluorescence intensity (fold)
150
100
50
0
Normal
B-LED
W-LED
W-CFL
Y-CFL
(n = 8 in each group)

A **ONL nucleolus**

Figure 6



B **Photoreceptors**

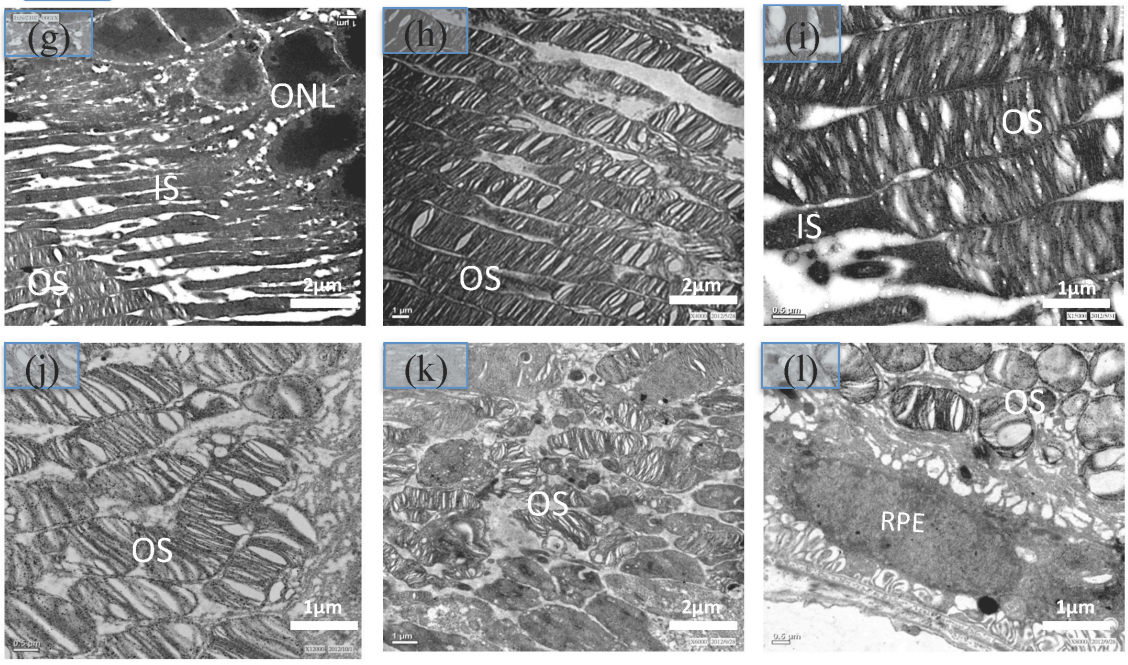


Figure 7

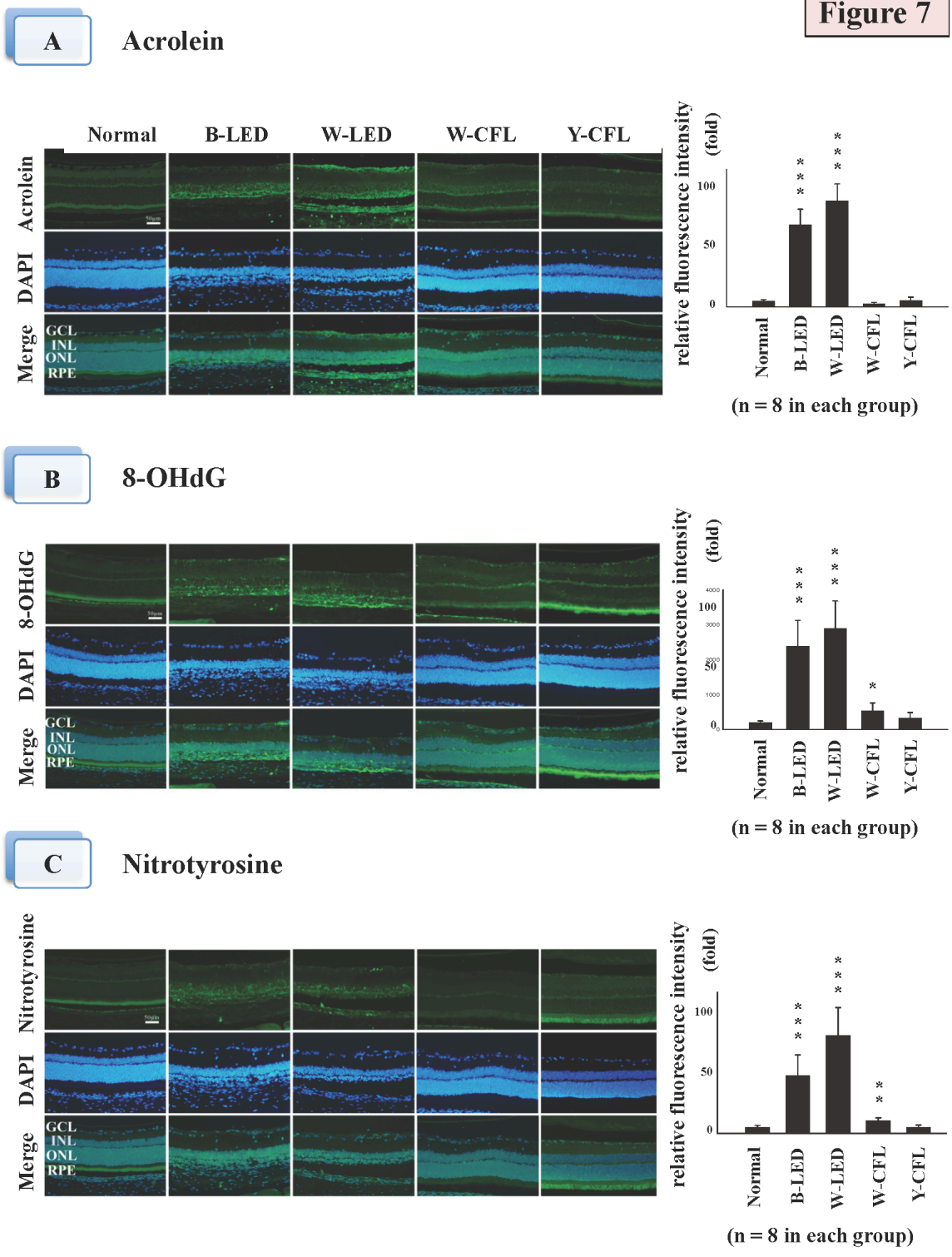
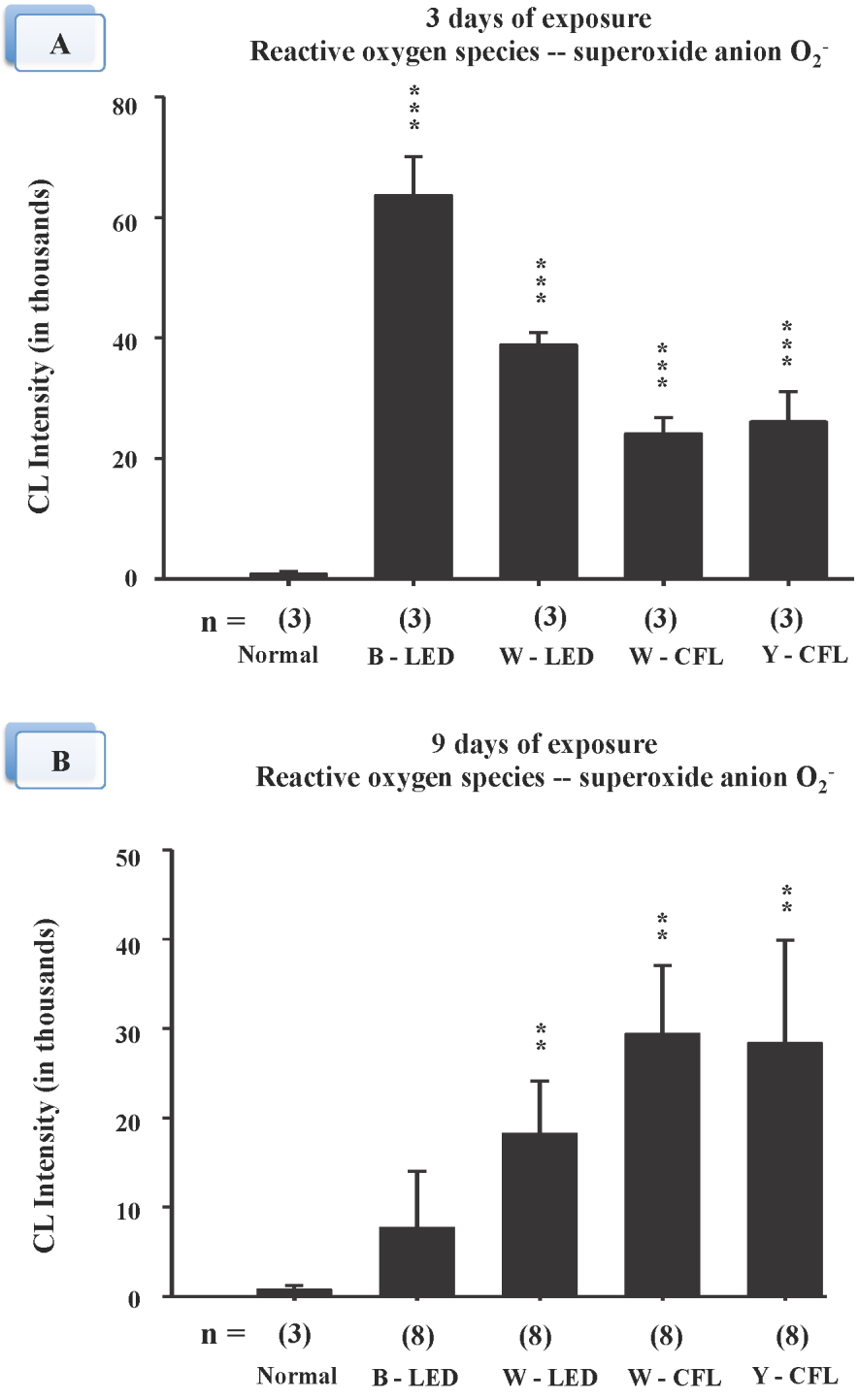

A
Acrolein
Normal
B-LED
W-LED
W-CFL
Y-CFL
Acrolein
DAPI
Merge
GCL
INL
ONL
RPE
relative fluorescence intensity (fold)
100
50
0
Normal
B-LED
W-LED
W-CFL
Y-CFL
(n = 8 in each group)
B
8-OHdG
8-OHdG
DAPI
Merge
GCL
INL
ONL
RPE
relative fluorescence intensity (fold)
4000
3000
2000
1000
0
Normal
B-LED
W-LED
W-CFL
Y-CFL
(n = 8 in each group)
C
Nitrotyrosine
Nitrotyrosine
DAPI
Merge
GCL
INL
ONL
RPE
relative fluorescence intensity (fold)
100
50
0
Normal
B-LED
W-LED
W-CFL
Y-CFL
(n = 8 in each group)

Figure 8


A
3 days of exposure
Reactive oxygen species -- superoxide anion O_2^-
CL Intensity (in thousands)
80
60
40
20
0
***
***
***
***
n = (3) (3) (3) (3) (3)
Normal
B - LED
W - LED
W - CFL
Y - CFL
B
9 days of exposure
Reactive oxygen species -- superoxide anion O_2^-
CL Intensity (in thousands)
50
40
30
20
10
0
**
**
**
**
n = (3) (8) (8) (8) (8)
Normal
B - LED
W - LED
W - CFL
Y - CFL